

WHAT IS CLAIMED IS:

1. A method for identifying a peptide-peptide interaction comprising:
- 5 (a) providing a first fusion construct comprising target peptide fused to a first DNA binding domain;
- (b) providing a second fusion construct comprising a library encoded peptide (LEP) fused to second DNA binding domain (DBD), wherein said second DBD works as a complex with said first DBD to facilitate binding of said complex to a prokaryotic operator region;
- 10 (c) contacting said first and second fusion constructs in a prokaryotic host cell which comprises said prokaryotic operator region, wherein said prokaryotic operator region is operationally linked to a coding region for one or more indicator polypeptides; and
- (d) determining binding of said complex to said operator region,
- 15 whereby binding of said complex to said operator region identifies said LEP as a binding partner for said target peptide.
2. The method of claim 1, wherein binding of said complex to said operator acts blocks the transcription of said coding region.
3. The method of claim 1, wherein said one or more indicator polypeptides render said prokaryotic host cell insensitive to phage infection.
- 20 4. The method of claim 3, wherein step (d) comprises infection with a phage that infects, replicates and lyses said prokaryotic host cell.
5. The method of claim 4, wherein said operator is the lacZ operator, and the first and second DBDs are derived from the λ repressor.

6. The method of claim 1, wherein one or more indicator polypeptides produce a colorimetric or fluorescent product.
7. The method of claim 1, wherein said one or more indicator polypeptides is β -gal.
8. The method of claim 1, wherein said target peptide is 5 to about 5000 residues in length.
9. The method of claim 1, wherein said target peptide is 10 to about 2000 residues in length.
10. The method of claim 1, wherein said LEP is 5 to about 50 residues.
11. The method of claim 1, wherein said first and second fusion constructs are encoded by a nucleic acid segment under the control of a promoter operable in said prokaryotic host cell.
12. The method of claim 1, wherein said target peptide and LEP bind with an affinity in the range of about 10^{-3} to about 10^{-6} M.
13. The method of claim 12, wherein said target peptide and LEP bind with an affinity in the range of about 10^{-4} M.
14. The method of claim 12, wherein said target peptide and LEP bind with an affinity in the range of about 10^{-5} M.
15. The method of claim 12, wherein said target peptide and LEP bind with an affinity in the range of about 10^{-6} M.
16. The method of claim 1, further comprising random mutagenesis of said LEP, followed by measuring the change, if any, in the binding affinity of said LEP for said target.
17. The method of claim 16, wherein said measuring comprises effecting binding of said LEP to said target peptide under conditions more stringent than in claim 1.

18. The method of claim 1, further comprising:
- (e) linking said identified LEP to a third peptide, whereby said linking permits said identified LEP and said third peptide to interact independently with said target peptide;
 - 5 (f) then contacting said target peptide with the identified LEP-third peptide complex, and
 - (g) followed by determining the change, if any, in the binding affinity of said LEP for said target peptide.
- 10 19. The method of claim 18, wherein said measuring comprises effecting binding of said LEP to said target peptide under conditions more stringent than in claim 1.
20. The method of claim 18, wherein said third peptide is known to bind said target peptide.
21. The method of claim 18, wherein said third peptide is a member of a peptide or peptidomimetic library.
- 15 22. The method of claim 1, wherein said target peptide is an enzyme substrate, an antigen, or a eukaryotic cell antigen.
23. The method of claim 22, wherein said target peptide is an enzyme substrate.
24. The method of claim 23, wherein said enzyme substrate is bacterial, viral or fungal antigen.
- 20 25. The method of claim 22, wherein said target peptide is a eukaryotic cell antigen.
26. The method of claim 25, wherein said eukaryotic cell antigen is a tumor cell marker, an HLA antigen, a cell surface receptor, or a cell surface transporter.
27. The method of claim 1, further comprising, prior to said determining, the step of stabilizing the interaction between said target peptide and said LEP.

28. The method of claim 27, wherein said stabilizing is achieved via cross-linking or phototrapping.

29. The method of claim 1, wherein said first peptide comprises a multimer of a smaller peptide unit.

5 30. The method of claim 1, further comprising assessing binding of said target peptide to said identified LEP by Western blot, mass spectroscopy, or nuclear magnetic resonance.

31.

A method for screening a peptide library for peptide-peptide interactions comprising:

10 (a) providing a plurality of a first fusion construct comprising a target peptide fused to a first DNA binding domain;

(b) providing a plurality of second fusion construct comprising a library of encoded peptide (LEPs) fused to second DNA binding domain (DBD), wherein said second DBD works as a complex with said first DBD to facilitate binding of said complex to a prokaryotic operator region;

15 (c) transferring said pluralities of first and second fusion constructs into a prokaryotic host cell which comprises said prokaryotic operator region, wherein said prokaryotic operator region is operationally linked to a coding region for one or more indicator polypeptides; and

20 (d) determining binding of complexes to said operator region,

whereby binding of said complexes to said operator region identifies associated LEPs as binding partners for said target peptide.

32. The method of claim 3, wherein steps (a)-(d) are repeated at least once using the LEP identified in step (d).

33. The method of claim 31, wherein said LEPs are synthesized from a four base cutter-digested DNA library.
34. The method of claim 33, further comprising the step of sequencing a DNA encoding an identified LEP.
- 5 35. A library encoded peptide (LEP) selected according to a method comprising:
- (a) providing a first fusion construct comprising target peptide fused to a first DNA binding domain;
 - (b) providing a second fusion construct comprising said LEP fused to second DNA binding domain (DBD), wherein said second DBD works as a complex with said first DBD to facilitate binding of said complex to a prokaryotic operator region;
 - (c) contacting said first and second fusion constructs in a prokaryotic host cell comprising said prokaryotic operator region, wherein said prokaryotic operator regions is operationally linked to a coding region for one or more indicator polypeptides; and
 - (d) determining binding of said complex to said operator region,
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- whereby binding of said complex to said operator region identifies said LEP as a binding partner for said target peptide.
36. A heterodimeric binding molecule comprising:
- (a) a first peptide that binds to a target molecule;
 - (b) a second peptide that binds to said target molecule,
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- wherein at least one of said first and second peptides is a member of a peptide library; and

(c) a linker molecule connecting said first and second peptides such that the linking permits said first and second peptides to interact independently with said target molecule.

5 37. The binding molecule of claim 36, further comprising a moiety that permits recovery of said molecule.

38. The binding molecule of claim 37, wherein said moiety is a magnetic bead.

39. The binding molecule of claim 36, wherein said heterodimeric binding molecule is expressed on the surface of a phage.

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